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CYP1A1 Val$_{462}$ and *NQO1 Ser*$_{187}$ polymorphisms, cigarette use, and risk for colorectal adenoma

Lifang Hou[1,*], Nilanjan Chatterjee[1], Wen-Yi Huang[1], Andrea Baccarelli[1], Sunita Yadavalli[1,3], Meredith Yeager[1,3], Robert S.Bresalier[4], Stephen J.Chanock[2], Neil E.Caporaso[1], Bu-Tian Ji[1], Joel L.Weissfeld[5] and Richard B.Hayes[1]

[1]Department of Human and Health Services, Division of Cancer Epidemiology and Genetics and [2]Section of Genomic Variation, Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, [3]SAIC-Frederick, National Cancer Institute, Frederick, MD, USA, [4]Department of Gastrointestinal Medicine and Nutrition, MD Anderson Cancer Center, Houston, TX, USA and [5]Department of Epidemiology, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

[*]To whom correspondence should be addressed at: National Cancer Institute, 6120 Executive Blvd, EPS 8123, Bethesda, MD 20892-7240, USA. Tel: +1 301 435 3348; Fax: +1 301 402 4489;
Email: lifangh2@mail.nih.gov

Cigarette use is a risk factor for colorectal adenoma, a known precursor of colorectal cancer. Polymorphic variants in *NQO1* and *CYP1A1* influence the activation of carcinogenic substances in tobacco smoke, possibly impacting on tobacco-associated risks for colorectal tumors. We investigated the association of cigarette smoking with risk for advanced colorectal adenoma in relation to the *CYP1A1 Val*$_{462}$ and *NQO1 Ser*$_{187}$ polymorphic variants. Subjects were 725 non-Hispanic Caucasian cases with advanced colorectal adenoma of the distal colon (descending colon, sigmoid and rectum) and 729 gender- and ethnicity-matched controls, randomly selected from participants in the prostate, lung, colorectal and ovarian cancer screening trial. Subjects carrying either *CYP1A1 Val*$_{462}$ or *NQO1 Ser*$_{187}$ alleles were weakly associated with risk of colorectal adenoma; however, subjects carrying both *CYP1A1 Val*$_{462}$ and *NQO1 Ser*$_{187}$ alleles showed increased risks (OR = 2.2, 95% CI = 1.1–4.5), particularly among recent (including current) (OR = 17.4, 95% CI = 3.8–79.8, *P* for interaction = 0.02) and heavy cigarette smokers (>20 cigarettes/day) (OR = 21.1, 95% CI = 3.9–114.4, *P* for interaction = 0.03) compared with non-smokers who did not carry either of these variants. These genotypes were unassociated with risk in non-smokers. In analysis of adenoma subtypes, the combined gene variants were most strongly associated with the presence of multiple adenoma (*P* = 0.002). In summary, joint carriage of *CYP1A1 Val*$_{462}$ and *NQO1 Ser*$_{187}$ alleles, particularly in smokers, was related to colorectal adenoma risk, with a propensity for formation of multiple lesions.

Abbreviations: AHH, aryl hydrocarbon hydroxylase; PLCO, prostate, lung, colorectal and ovarian; ROS, reactive oxygen species.

Introduction

Colorectal adenoma is the major precursor of colorectal cancer (1,2). Given the high prevalence of adenomas and the proportional rarity of colorectal cancers (3), identifying determinants of high-risk adenoma may facilitate the development of preventive strategies to colorectal cancer. Cigarette smoking increases risk for colorectal adenoma, and probably also for colon cancer, at least among the long-term smokers (4). Most chemical carcinogens in cigarette smoke require metabolic activation by phase I enzymes such as P450 enzymes and detoxification by phase II enzymes (5–8). Metabolic activation of polycylic aromatic hydrocarbons PAHs by cytochrome P450 enzymes leads to oxidized derivatives, including quinones (9,10), resulting in large amounts of reactive oxygen species (ROS), which damage DNA (6,10,11).

CYP1A1 is a phase I metabolic enzyme which encodes the aryl hydrocarbon hydroxylase (AHH) enzymes responsible for the activation of a range of chemical carcinogens, including benzo[*a*]pyrene (B[*a*]P) and other polycyclic aromatic hydrocarbons (PAHs) (12). In contrast, NQO1 protects cells against toxicity by catalyzing the two-electron reduction and detoxification of quinones to hydroquinones, impeding the formation of DNA-quinone adducts (5,13,14). Thus, the coordinated expression and regulation of CYP1A1 and NQO1 enzymes and their enzymatic balance may determine the extent of cellular DNA damage and related development of cancer (15). *CYP1A1 Val*$_{426}$ carriers exhibit higher levels of CYP1A1 enzymatic activity and inducibility, particularly in smokers (16), and smokers who carry this variant also have increased peripheral white blood cell PAH–DNA adducts (17). However, differential enzymatic activity has not been consistently shown *in vitro* (18,19) and involvement of *CYP1A1 Val*$_{426}$ allele in several tobacco-related cancers is controversial.

The heterozygous and homozygous carriers of *NQO1 Ser*$_{187}$ allele are reported to have an ~3-fold decreased and completely null NQO1 enzyme activity, respectively, toward carcinogens present in tobacco smoke (6,7,20,21). Increased *NQO1 Ser*$_{187}$ allele frequency is also reported among cancer patients, including those with lung, bladder, and esophageal cancer and leukemia (15,22–24).

Although tobacco use is related to colorectal adenoma development, epidemiologic studies have not shown clear associations of either the *CYP1A1 Val*$_{462}$ or *NQO1 Ser*$_{187}$ alleles with colon cancer or colorectal adenoma (25). We investigated the roles of these genetic variants in *CYP1A1* and *NQO1,* in relation to tobacco use, in an early detection trial of cancer. The study focuses on advanced adenoma, i.e. tumors with greater potential for malignant transformation.

Methods

The PLCO Trial

The National Cancer Institute (NCI) prostate, lung, colorectal and ovarian (PLCO) cancer screening trial randomized 77 483 screening arm participants

(38 364 men, 39 119 women) and a similar number of non-screened controls, aged 55–74, at 10 US screening centers (Birmingham AL, Denver CO, Detroit MI, Honolulu HI, Marshfield WI, Minneapolis MN, Pittsburgh PA, Salt Lake City UT, St Louis MO and Washington DC) (26). At study entry, flexible sigmoidoscopic visualization of the distal colon (60 cm) was done on participants in the screening group. If the sigmoidoscopic examination was suspicious for neoplasia (polyp or mass), the screenees were referred for endoscopic follow-up, including histopathologic examination. All available pathology reports on the removed lesions were obtained and coded by trained medical abstractors (location, size, morphology, etc.). Questionnaire data and biologic samples were acquired from study participants (27). Participants provided written informed consent. The study was approved by the institutional review boards of the NCI and the 10 screening centers.

Selection of study subjects

The investigation is a part of the NCI- PLCO cancer screening trial. Subject selection has been described in detail elsewhere (28,29); in brief, cases and controls were drawn from screening-arm participants at the 10 PLCO trial screening centers between September 1993 and September 1999, who filled out risk-factor questionnaires, had a successful sigmoidoscopy (insertion up to at least 50 cm with >90% of mucosa visible or a suspect lesion identified), and provided a blood sample for use in etiologic studies ($n = 42\,037$). We further excluded 4834 subjects with a self-reported history of ulcerative colitis, Crohn's disease, familial polyposis, colorectal polyps, Gardner's syndrome or cancer (except for basal cell skin cancer). With a goal of including ~800 cases and 800 controls, we randomly selected 772 of 1234 cases with at least one advanced colorectal adenoma (adenoma $\geq$1 cm or containing high-grade dysplasia or villous elements, including tubulovillous elements) in the distal colon (descending colon and sigmoid or rectum) and 777 of 26 651 control participants, with a negative sigmoidoscopy screening (i.e. no polyp or other suspect lesion), frequency-matched to the cases by gender and ethnicity (Non-Hispanic White, Non-Hispanic Black, Hispanic and others). For this investigation, we studied only Non-Hispanic Whites (94% of total subjects, including 725 cases of advanced adenoma and 729 controls) owing to the small number of subjects with other ethnic/racial backgrounds and the significant heterogeneity in *CYP1A1* Val_{462} allele frequencies between Non-Hispanic Whites and other ethnic groups ($P < 0.001$).

Questionnaire data

Participants completed baseline general risk factor and food frequency questionnaires, reporting information on demographic characteristics, including education, race and marital status, first-degree family history of cancer, body size, use patterns of tobacco, alcohol consumption, selected drugs and hormones, and the usual dietary intake over the 12 months prior to enrolment. Detailed information on smoking history was collected, including ages started and stopped, number of years of tobacco used, amount usually consumed and the type of tobacco used (cigarettes, pipes or cigars). Individuals who did not smoke cigarettes for >6 months and did not smoke cigars or pipe for more than a year were considered non-smokers. Subjects who used cigars or pipe for 1 year or more, but did not smoke cigarettes for >6 months, were considered as cigars/pipe users only.

Preliminary analyses of the PLCO study showed increased risks for advanced colorectal adenoma among subjects who were current smokers or had quit within 20 years before the study (30). To assess time-dependent effects in adenoma risk, we contrasted risks between never smokers, smokers who had quit 20 years or longer and smokers who were current users or had quit in the last 19 years. To assess dose effects, we contrasted never users, with smokers who consumed <20 cigarettes per day and smokers of $\geq$20 cigarettes per day. Dietary nutrient intake was calculated by multiplying the reported frequency of consumption for relevant food items by gender- and nutrient-specific portion size (31), using the nutrient database from the US Department of Agriculture (32). Dietary intake of B[*a*]P was calculated using a database developed by Kazerouni *et al*. (33).

Genotyping genetic variants

Genotyping of the two common variants, *CYP1A1* ($Ile_{462}Val$) and *NQO1* ($Pro_{187}Ser$), was performed by TaqMan™ assay (Applied Biosystems, Inc., Foster City, CA) using a 384-well plate and analyzed on an ABI 7900HT sequence detection system, plotted with SDS software. Assays were validated and optimized as described in the SNP500 Cancer website (http://snp500cancer.nci.nih.gov). Assay-specific primer/probe concentrations and thermo-cycling conditions are also available there for the *CYP1A1*-01 (rs# 1048943) and *NQO1*-01 (rs# 1800566) assays. Internal laboratory quality controls included four of each of the Coriell DNA samples containing homozygous major allele, and heterozygous and homozygous minor allele genotypes for each polymorphism under study and four no template controls in every 384 samples. Approximately 10% blinded quality control samples from 40 individuals were interspersed with the study samples, showing >99% concordance. Genotype data were successfully obtained for 91% (*CYP1A1* $Ile_{462}Val$) and 90% (*NQO1* $Pro_{187}Ser$) of the study subjects. Individuals with insufficient DNA (7%), genotyping failures (0.9% for *CYP1A1* $Ile_{462}Val$ and 4% for *NQO1* $Pro_{187}Ser$) were excluded from the study. We also dropped subjects (0.6%) from analyses who had ambiguous genetic profiles, based on our standard quality control with 16 highly polymorphic markers. After exclusion of genotype failures, 700 cases and 708 controls were available for *NQO1* ($Pro_{187}Ser$) analysis and 675 cases and 679 controls were available for *CYP1A1* ($Ile_{462}Val$) analysis. The combined genotype status was examined in 668 cases and 668 controls.

Statistical analysis

Genotypes for *CYP1A1* $Ile_{462}Val$ and *NQO1* $Pro_{187}Ser$ *were assigned* the scores: 0, no variant allele; 1, carrying one variant allele (Val_{462} for *CYP1A1* or Ser_{187} for *NQO1*); and 2, carrying two variant alleles. Hardy–Weinberg equilibrium was tested using the asymptotic Pearson's χ^2-test. Odds ratios (ORs) for adenoma risk and 95% confidence intervals (CIs) were calculated using unconditional logistic regression. The regression coefficient corresponding to the integer score provides an overall measure of strength of association and is reported as the trend statistic.

The statistical significance of a multiplicative interaction term was tested using the likelihood ratio test, comparing logistic regression models with and without the appropriate interaction term. For testing interaction between a two-level genotype variable (absence/presence of variant alleles) and a three-level smoking variable, we included the smoking variable in the logistic regression model as categorical for the main effect terms, and as ordinal (integer score) for the interaction term; the corresponding χ^2-test for interaction has one degree of freedom.

We obtained estimates of adenoma risks by joint status of *CYP1A1* Val_{462}, *NQO1* Ser_{187} and smoking. We present the results from logistic regression models that included terms for the main effects and all second-order interaction terms involving *CYP1A1* Val_{462} genotype, *NQO1* Ser_{187} genotype and smoking status. Logistic regression models that also included explicit terms describing the three-way gene–gene–smoking interaction were unstable. The test for gene–gene–smoking interaction was performed by comparing subjects who had both *CYP1A1* (Val_{462}) and *NQO1*(Ser_{187}) alleles against those who did not carry either of the variant alleles, and considering cigarette use as an

Table I. Description of study subjects

Characteristics	Controls $N = 729$ *N* (%)	Cases $N = 725$ *N* (%)
Sex		
Male	502 (68.8)	505 (69.7)
Female	227 (31.2)	220 (30.3)
Age at interview		
55–59 years	332 (45.5)	241 (33.3)
60–64 years	192 (26.3)	225 (31.0)
65–69 years	137 (18.8)	162 (22.3)
70–74 years	68 (9.34)	97 (13.4)
First degree family history of colorectal cancer		
No	664 (91.1)	637 (87.7)
Yes	65 (8.9)	89 (12.3)
Level of education		
$\leq$11 years	41 (5.6)	63 (8.7)
12 years or high school	170 (23.4)	182 (25.1)
Some college[a]	233 (32.0)	258 (35.7)
College and above	284 (39.0)	221 (30.5)
Cases of advanced adenoma[b]		
Size of adenoma		
<1 cm	—	125 (17.2)
$\geq$1 cm	—	536 (74.0)
Unknown	—	64 (8.8)
Multiplicity		
Single	—	497 (68.6)
Multiple	—	228 (31.4)
Advanced histology[c]		
No	—	264 (36.4)
Yes	—	461 (63.6)

[a]Post-high school and some college education.
[b]Case numbers are not mutually exclusive.
[c]Adenomas with high-grade dysplasia or villous elements.

ordinal variable (coded: 0,1,2: 0, non-smokers, 1, quit smoking ≥20 years or smoked ≤20 cigarettes/day; and 2, quit smoking <20 years or smoked >20 cigarettes/day).

Our effort was to study whether the joint effect of *CYP1A1* and *NQO1* polymorphisms varied by three different characteristics of adenoma: size (≥1 cm versus <1 cm), multiplicity (multiple versus single) and presence of advanced histology. Based on a novel extension of polytomous logistic regression (34), we examined heterogeneity in the effect of the alleles by case–case OR parameter defined by each individual characteristics (comparing one subtype of case with another, e.g. large versus small adenoma), after controlling for the other two characteristics (e.g. multiplicity and histology). Adjustment for first-degree family history of colorectal cancer, education, body mass index and dietary intake of fiber and red meat did not substantially alter the results, and were not included in the analyses presented here. Age was weakly correlated with disease status and was included as a covariate along with gender in the statistical analyses. All *P*-values were two-sided. Individuals with missing values were excluded from specific analyses. All analyses were calculated using Stata (Stata Corporation, College Station, TX; version 8.0) and MATLAB (Mathwork Inc.; version 5.3.1).

Results

Gender was similar for both cases and controls (Table I). Cases tended to be older ($P < 0.001$), more likely to report a first-degree family history of colorectal cancer ($P = 0.04$) and were less educated ($P = 0.001$). Among the 725 cases, 536 (74.0%) had a lesion ≥1 cm, 461 (63.6%) showed advanced histological features and 228 (31.4%) had multiple adenoma.

Compared with non-smokers, risks for advanced adenoma were significantly increased among current and recent smokers (i.e. quit within the last 20 years) (OR = 1.9, 95% CI = 1.5–2.4) and among those who smoked ≥20 cigarettes per day (OR = 1.7, 95% CI = 1.3–2.3) (Table II). Among cigar or pipe only users, no increased risk was observed. Dietary intake of B[*a*]P did not alter the risk (data not shown).

Among controls, both *CYP1A1* (*$Ile_{462}Val$*) and *NQO1* (*$Pro_{187}Ser$*) genotype distributions were in Hardy–Weinberg equilibrium in whites. The *NQO1 Ser_{187}* allele frequency was 0.18, similar to that reported (0.20) in a pooled analysis of other studies (25). The *CYP1A1 Val_{462}* allele frequency was 0.03, lower than reported (0.10) in the same pooled analysis (25). The *CYP1A1 Val_{462}* variant by itself was not associated with adenoma risk (Table II), while the *NQO1 Ser_{187}* variant showed a marginal association (OR = 2.0, 95% CI = 1.0–4.0 for those carrying two *NQO1 Ser_{187}* variants, *P* trend = 0.09, compared with those carrying none). Subjects carrying both *CYP1A1 Val_{462}* and *NQO1 Ser_{187}* variants had a significantly elevated risk (OR = 2.2, 95% CI = 1.1–4.5, *P* for interaction = 0.02).

Compared with non-smoking non-carriers, recent smokers who carried at least one copy of *CYP1A1 Val_{462}* allele

Table II. Risk of colorectal adenoma by smoking and genotype status[a]

	Controls (*N* = 729) *N* (%)	Cases (*N* = 725) *N* (%)	OR (95% CI)[b]	P_{trend}
Smoking status				
Non-smokers	296 (40.8)	245 (33.9)	1.0 (Referent)	
Smokers				
Cigar or pipe only	42 (5.8)	35 (4.9)	1.1 (0.6–1.8)	
Cigarette	391 (53.4)	445 (61.2)	1.4 (1.1–1.8)	
Quit ≥20 years	194 (26.7)	164 (22.7)	1.2 (0.8–1.3)	
Quit <20 years[c]	194 (26.7)	278 (38.5)	1.9 (1.5–2.4)	
Trend statistic[d]			*1.4 (1.2–1.6)*	<0.001
≤20 cigarettes/day	235 (32.1)	238 (32.7)	1.3 (1.0–1.6)	
>20 cigarettes/day	156 (21.3)	207 (28.5)	1.7 (1.3–2.3)	
Trend statistic[d]			1.3 (1.1–1.5)	<0.001
CYP1A1 Val_{462}				
0	643 (94.7)	633 (93.8)	1.0 (Referent)	
1	36 (5.3)	40 (5.9)	1.1 (0.7–1.9)	
2	0 (0)	2 (0.3)	—	—
1–2[e]	36 (5.3)	42 (6.2)	1.1 (0.7–1.8)	
Trend statistic[f]			1.3 (0.9–1.9)	0.23
NQO1 Ser_{187}				
0	468 (66.1)	435 (62.1)	1.0 (Referent)	
1	228 (32.2)	243 (34.7)	1.1 (0.9–1.4)	
2	12 (1.7)	22 (3.2)	2.0 (1.0–4.0)	
1–2[e]	240 (33.9)	265 (37.9)	1.2 (0.9–1.4)	
Trend statistic[f]			1.2 (0.98–1.5)	0.09
Joint effect of *CYP1A1* and *NQO1*				
CYP1A1 Val_{462} *NQO1 Ser_{187}*				
0 0	419 (62.7)	400 (59.8)	1.0 (Referent)	
0 1–2	214 (32.0)	227 (34.0)	1.1 (0.9–1.4)	
1–2 0	23 (3.5)	15 (2.2)	0.6 (0.3–1.2)	
1–2 1–2	12 (1.8)	26 (4.0)	2.2 (1.1–4.5)	
$P_{interaction}$[g]			0.02	

[a]Some numbers may not add up to the total, owing to missing values.
[b]Adjusted for age and sex.
[c]Includes current smokers.
[d]OR for smoking status as an ordinal variable 0 (non-smokers), 1 (quit smoking ≥20 years or smoked ≤20 cigarettes per day) and 2 (quit smoking <20 years or smoked 20 cigarettes per day).
[e]Carrying at least one *CYP1A1 Val_{462}* or *NQO1 Ser_{187}* allele.
[f]OR for the number of alleles, as an ordinal variable: 0, no variant allele; 1, one variant (*Val_{462}* for *CYP1A1* or *Ser_{187} for NQO1)*; 2, two variants.
[g]*P* for interaction between two genes was obtained using ordinal variables.

Table III. Risk of colorectal adenoma by recency of cigarette use and genotype status[a]

		Non-smokers			Past smokers[c] (quit ≥20 years)			Recent (quit <20 years) and current smokers[c]			Trend statistic[d]
		Controls (%)	Cases (%)	OR (95% CI)[b]	Controls (%)	Cases (%)	OR (95%CI)[b]	Controls (%)	Cases (%)	OR (95%CI)[b]	
CYP1A1 (Val_{462})											
0		258 (93.1)	219 (96.0)	1.0 (Referent)	172 (95.0)	144 (92.9)	1.0 (0.7–1.3)	172 (96.6)	243 (93.1)	1.9 (1.4–2.4)	1.4 (1.2–1.6)
1–2		19 (6.9)	9 (4.0)	0.6 (0.2–1.3)	9 (5.0)	11 (7.1)	1.3 (0.5–3.3)	6 (3.4)	18 (6.9)	3.8 (1.5–9.9)	2.1 (1.1–4.2)
$P_{interaction}$										0.03	
NQO1 (Ser_{187})											
0		189 (66.5)	158 (67.5)	1.0 (Referent)	128 (67.0)	99 (62.7)	0.9 (0.6–1.3)	124 (65.6)	158 (58.1)	1.7 (1.2–2.3)	1.3 (1.1–1.5)
1–2		95 (33.5)	76 (32.5)	0.9 (0.6–1.3)	63 (33.0)	59 (37.3)	1.1 (0.7–1.6)	65 (34.4)	114 (41.9)	2.2 (1.5–3.2)	1.6 (1.3–2.0)
$P_{interaction}$										0.17	
CYP1A1 (Val_{462})	*NQO1* (Ser_{187})										
0	0	170 (63.2)	149 (67.4)	1.0 (Referent)	113 (63.1)	93 (60.4)	0.9 (0.7–1.3)	110 (62.2)	140 (54.3)	1.6 (1.1–2.3)	1.2 (1.0–1.5)
0	1–2	81 (30.1)	64 (29.0)	0.9 (0.6–1.3)	57 (31.8)	50 (32.5)	1.0 (0.6–1.5)	61 (34.4)	100 (38.7)	2.0 (1.3–3.0)	1.6 (1.3–2.0)
1–2	0	10 (3.7)	1 (0.5)	0.2 (0.05–0.6)	7 (3.9)	4 (2.6)	0.5 (0.2–1.7)	5 (2.8)	8 (3.1)	1.9 (0.7–5.6)	3.6 (0.8–15.3)
1–2	1–2	8 (3.0)	7 (3.1)	0.9 (0.3–2.5)	2 (1.1)	7 (4.5)	4.1 (1.1–14.9)	1 (0.6)	10 (3.9)	17.4 (3.8–79.8)	5.5 (1.3–23.9)
$P_{interaction}$[e]										0.02	

[a]Some numbers may not add up to the total due to missing values.
[b]Adjusted for age and sex.
[c]Subjects who never used cigarettes, but used pipes or cigars for 1 year were excluded from this analysis.
[d]Trend statistics within each genotype subgroup for recency of cigarette use (non-smokers = 0, past smokers (quit ≥ 20 years) = 1, recent (quit<20 years) and current smokers = 2, treated as an ordinal variable).
[e]*P* value for interaction between both *CYP1A1* Val_{462} and *NQO1* Ser_{187} variant alleles present (1–2, 1–2) versus no variant alleles (0, 0) and daily cigarette use (ordinal variable, coded as 0, 1, 2).

Table IV. Risk of colorectal adenoma by daily cigarette use and genotype status[a]

		Non-smokers			≤20 cigarettes/day[c]			>20 cigarettes/day[c]			Trend statistic[d]
		Controls (%)	Cases (%)	OR (95%CI)[b]	Controls (%)	Cases (%)	OR (95%CI)[b]	Controls (%)	Cases (%)	OR (95%CI)[b]	
CYP1A1 (Val_{462})											
0		258 (93.1)	219 (96.0)	1.0 (Referent)	207 (94.5)	215 (94.3)	1.3 (1.0–1.7)	140 (97.9)	175 (91.6)	1.6 (1.2–2.2)	1.3 (1.1–1.5)
1–2		19 (6.9)	9 (4.0)	0.6 (0.2–1.3)	12 (5.5)	13 (5.7)	1.2 (0.6–2.9)	3 (2.1)	16 (8.4)	6.4 (1.8–22.3)	2.9 (1.3–6.6)
$P_{interaction}$										0.007	
NQO1 (Ser_{187})											
0		189 (66.5)	158 (67.5)	1.0 (Referent)	159 (69.7)	147 (62.8)	1.1 (0.8–1.5)	95 (61.3)	111 (57.8)	1.5 (1.1–2.2)	1.2 (1.0–1.4)
1–2		95 (33.5)	76 (32.5)	0.9 (0.6–1.3)	69 (30.3)	87 (37.2)	1.5 (1.0–2.2)	60 (38.7)	88 (44.2)	1.8 (1.2–2.7)	1.5 (1.2–1.9)
$P_{interaction}$										0.32	
CYP1A1 (Val_{462})	*NQO1* (Ser_{187})										
0	0	170 (61.8)	149 (65.9)	1.0 (Referent)	141 (65.3)	136 (49.1)	1.1 (0.8–1.6)	83 (58.0)	99 (52.6)	1.4 (1.0–2.1)	1.2 (1.0–1.4)
0	1–2	87 (31.6)	69 (30.5)	0.9 (0.6–1.3)	63 (29.2)	78 (55.3)	1.4 (0.9–2.1)	56 (39.1)	74 (39.4)	1.6 (1.1–2.4)	1.4 (1.1–1.8)
1–2	0	10 (3.7)	1 (0.5)	0.2 (0.05–0.6)	9 (4.2)	6 (40.0)	0.7 (0.2–2.1)	3 (0.2)	6 (3.2)	3.0 (0.8–11.7)	3.2 (0.9–11.6)
1–2	1–2	8 (2.9)	7 (3.1)	0.9 (0.3–2.5)	3 (1.3)	7 (70.0)	4.4 (1.2–15.7)	1 (0.07)	9 (4.8)	21.1 (3.9–114.4)	10.6 (1.6–68.5)
$P_{interaction}$[e]										0.03	

[a]Some numbers may not add up to the total due to missing values.
[b]Adjusted for age and sex.
[c]Subjects who never used cigarettes, but used pipes or cigars for ≥1 year were excluded from this analysis.
[d]Trend statistics within each genotype subgroup for daily cigarette use (non-smokers = 0, ≤20 cigarettes per day = 1, >20 cigarettes per day = 2, treated as an ordinal variable).
[e]*P* value for interaction between both *CYP1A1* Val_{462} and *NQO1* Ser_{187} variant alleles present (1–2, 1–2) versus no variant alleles (0, 0) and daily cigarette use (ordinal variable, coded as 0, 1, 2).

Table V. Case–case OR[a] (95% CI) for subjects with *CYP1A1*(Val_{462}) and *NQO1*(Ser_{187}) alleles, by selected pathological characteristics[b,c]

Pathological characteristics[d]	Number of *CYP1A1*(Val_{462}) and *NQO1*(Ser_{187}) alleles					
	CYP1A1 (Val_{462})	*NQO1* (Ser_{187})	*CYP1A1* (Val_{462})	*NQO1* (Ser_{187})	*CYP1A1* (Val_{462})	*NQO1* (Ser_{187})
	1–2	0	0	1–2	1–2	1–2
	OR (95%CI)		OR (95%CI)		OR (95%CI)	
Size[e]						
Large versus small	1.2 (0.7–1.9)		1.0 (0.2–5.7)		0.3 (0.1–1.0)	
Advanced histology						
Yes versus no	1.6 (1.1–2.3)		0.9 (0.3–3.6)		0.4 (0.1–1.2)	
Multiplicity						
Multiple versus single	1.3 (0.9–1.9)		1.0 (0.3–3.8)		4.1 (1.7–10.2)	

[a]Adjusted for age sex and smoking, with analysis for each tumor characteristic controlled for the distribution of the other two tumor characteristics. Referent genotype group: no variant alleles (0, 0) in both genes.
[b]Subjects who never used cigarettes, but used pipes or cigars for ≥1 year were excluded from this analysis.
[c]Some numbers may not add up to the total due to missing values.
[d]Case numbers by tumor type are not mutually exclusive.
[e]Sixty-four subjects with unknown adenoma size were excluded.

(OR = 3.8, 95% CI = 1.5–9.9) and at least one *NQO1* Ser_{187} allele (OR = 2.2, 95% CI = 1.5–3.2) were at an increased risk of adenoma. Subjects who smoked recently and carried both *CYP1A1* Val_{462} and *NQO1* Ser_{187} had the greatest risk (OR = 17.4, 95% CI = 3.8–79.8), compared with non-smokers lacking *CYP1A1* Val_{462} and *NQO1* Ser_{187} alleles (Table III). Statistical tests for interaction showed greater increases in risk with recency of cigarette use among *CYP1A1* Val_{462} carriers (P for interaction = 0.03) and among carriers of both *CYP1A1* Val_{462} and *NQO1* Ser_{187} (P for interaction = 0.02).

Similar patterns were noted when daily cigarette use was considered (Table IV). *CYP1A1* Val_{462} carriers who smoked ≥20 cigarettes per day showed significantly increased risks (OR = 6.4, 95% CI = 1.8–22.3, P for interaction = 0.007), compared with non-smoking non-carriers of *CYP1A1* Val_{462}. Subjects who smoked ≥20 cigarettes per day and carried both *CYP1A1* Val_{462} and *NQO1* Ser_{187} had the greatest risks, compared with non-smoking non-carriers (OR = 21.1, 95% CI = 3.9–114.4, P for interaction is 0.03).

Analysis of tumor type specific risks showed that the combination of the *CYP1A1* Val_{462} and *NQO1* Ser_{187} variants together was more strongly associated with multiple adenoma compared with single adenoma (OR = 4.1, 95% CI = 1.7–10.2, P = 0.002), and tended to be associated with non-advanced histology (for advanced tumors, OR = 0.4, 95% CI = 0.1–1.2, P = 0.07) and smaller tumor size (for large tumors, OR = 0.3, 95% CI = 0.1–1.0, P = 0.05) (Table V). No such variation in risk was observed for the combinations of genotypes indicating carriage of one or the other variant only.

Discussion

We found that smoking-associated risks for advanced colorectal adenoma tended to be greatest in *CYP1A1* Val_{462} and *NQO1* Ser_{187} carriers, particularly those who carried both gene variants, and that these genetic risks were not seen in non-smokers. The evidence for the association between cancer at different sites with either *CYP1A1* (*I462V*) or *NQO1* (*P187S*) polymorphisms has been controversial (15,16,21–25,35–41). A recent pooled analysis, including four studies of *CYP1A1* Val_{462} (460 cases and 742 controls), and two studies of *NQO1* Ser_{187} (570 cases and 501 controls), showed no clear overall associations between the *CYP1A1* Val_{462} or *NQO1* Ser_{187} variants and risk of colorectal cancer (25). However, of the six studies, only two considered the inter-relationships between genetic polymorphisms and tobacco use (37,42), with one suggesting protective effects of NQO1 Pro_{187} among smokers (OR = 0.434, 95% CI = 0.13–1.2) (37).

NQO1 protects cells from carcinogens in cigarette smoke through competition with CYP1A1 for quinone substrates, inhibiting the formation of CYP1A1-generated metabolites and subsequent binding to DNA (14). *NQO1* Ser_{187} allele has been linked to decreased NQO1 enzyme activity (6,7,20,21) and *CYP1A1* Val_{426} allele carriers were reported to have higher levels of *CYP1A1* enzymatic activity and inducibility, particularly in smokers (16). Thus, the presence of both the *CYP1A1* variant related to increased metabolism to toxic compounds and the *NQO1* variant related to decreased detoxification may result in greater doses in smokers of B[*a*]P reactive metabolites at the cellular level, yielding increased risks for B[*a*]P-associated diseases.

Using a recently developed statistical approach to evaluate the impact of risk factors on one of several overlapping disease characteristics (34), we found that carrying both *CYP1A1* Val_{462} and *NQO1* Ser_{187} variants was most strongly associated with risks for multiple, compared with single adenomas, and possibly to small, histologically less aggressive lesions. This is consistent with previous studies showing a relationship between tobacco use and adenoma multiplicity (43,44) and with data indicating that cigarette use is most strongly linked to colorectal cancer precursors than to cancer itself (45,46). Although the underlying biological mechanism for this observation is still unknown, B[*a*]P metabolites of these enzymes may particularly influence the early stages of the adenoma–carcinoma sequence, i.e. the transition from normal colonic mucosa to early adenoma. Therefore, we speculate that the metabolic pathway with involvement of both CYP1A1 and NQO1 is more involved in early colorectal carcinogenesis, rather than the later development of large or more histologically aggressive tumors.

The large sample size in our study allowed us to examine inter-relationships of *CYP1A1* Val_{462}, *NQO1* Ser_{187} and smoking and to explore associations with specific adenoma

sub-types. Further study is needed to define these relationships for colorectal cancer and to examine risks in other population and ethnic groups.

Our study of more than 725 cases and 729 controls, selected from a colorectal cancer screening study, showed that *CYP1A1 Val*$_{426}$ and *NQO1 Ser*$_{187}$ carriers who smoke are at increased risk for colorectal adenoma, particularly for multiple colorectal adenoma. The findings point to involvement of B[*a*]P metabolic pathways in colorectal carcinogenesis.

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